ON THE PHYLOGENETIC SIGNIFICANCE OF SPERMATOZOAL MORPHOLOGY AND MALE REPRODUCTIVE TRACT ANATOMY IN AUSTRALIAN RODENTS

by W. G. BREED* & V. SARAFIS†

Summary

BREED, W. G. & SARAFIS, V. (1978) On the phylogenetic significance of spermatozoal morphology and male reproductive tract anatomy in Australian rodents. *Trans. R. Soc. S. Aust.* **103**(5), 127-135, 31 August, 1979.

Spermatozoa of *Pseudomys nanus, P. hermannsburgensis, P. higginsi, P. australis, P. apodemoides, Leporillus conditor, Uromys caudimaculatus, Melomys littoralis, M. vervinipes* and *Conilurus penicillatus* are similar, having a head with three hooks and very prominent midpieces. In *Zyzomys argurus, Z. woodwardi* and *Hydromys chrysogaster* only two hooks could be seen. Spermatozoa morphology of *Notomys* sp. is variable. *N. alexis* had a short top hook and small, truncated, lower hook, whereas the sperm of *N. mitchellii* were either similar with a longer top hook or had three short straight hooks. All *Rattus* species had spermatozoa with a single much longer and more attenuated hook and a longer midpiece.

The morphology of the male reproductive tracts of *P. australis, Z. argurus, M. littoralis, Rattus fuscipes* and *H. chrysogaster* is similar. Testes lie in scrotal sacs and large seminal vesicles are present. By contrast, the morphology of the reproductive tracts of *Notomys* species is considerably different: their testes are smaller, usually naturally cryptorchid, and seminal vesicles are barely visible to the naked eye although large ventral prostates occur. The phylogenetic implications of the findings are discussed.

Introduction

There is controversy over the phylogenetic relationships of Australian native rodents (Tate 1951; Simpson 1961; Watts 1974; Baverstock *et al.* 1977b; Baverstock *et al.* 1977c) although all species are considered members of the Muridae.

On the basis of a wealth of morphometric data, Tate (1951) classified the Australopapuan rodents into two subfamilies: the Hydromyinae, which he considers diverged from an ancestral murid or even cricetid stock and is represented in Australia by *Xeromys* and *Hydromys*, and the Murinae which includes all other genera. In the Murinae he considers that *Pseudomys, Leporillus, Mastacomys, Notomys, Zyzomys* and *Conilurus* evolved from one ancestral stock, whereas a more modern group branched off from a stem leading to *Rattus* and gave rise to *Melomys* and *Uromys*. Simpson (1961) identified four groups, two subfamilies (the Hydromyinae and Pseudomyinae) and two other groups: one of *Rattus* species and the other of *Uromys/Melomys*. Of these the Pseudomyinae, which includes *Notomys, Conilurus, Pseudomys* and *Leporillus* species, as well as several other genera, has radiated mainly in Australia, whereas the other three groups are well represented in New Guinea.

Several authors have recently hypothesised phylogenetic relationships. Watts (1974) put forward a phylogenetic scheme in which *Pseudomys* and *Rattus* are closely related and *Pseudomys* was considered ancestral to all Australian rodents with the exception of *Melomys, Mastacomys* and *Rattus*. *Mastacomys* is shown diverging early from the ancestral stock, as is *Rattus* and *Pseudomys*. As a result of chromosomal analysis Baverstock *et al.* (1977b, 1977c) concluded that *Rattus* stood out as a distinct group with the Hydromyinae, and the *Uromys/Melomys* group diverged at an early stage from the ancestral stock which gave rise to the Pseudomyinae. The position of *Zyzomys* was considered enigmatic, but they

* Department of Anatomy and Histology, University of Adelaide, G.P.O. Box 498, Adelaide, S. Aust. 5001.
† Department of Biology, Hawkesbury Agricultural College, Richmond, N.S.W. 2753.

considered that it is probably derived from the same ancestral stock that gave rise to the Pseudomyinae.

Baverstock *et al.* (1977b) concluded that sperm morphology might be a particularly useful character in gaining further evidence about the phylogeny of Australian rodents, as sperm are less likely to be related to the lifestyle of the animal than morphological characters. In this study, therefore, we present data on spermatozoal morphology, together with some other aspects of the male reproductive tract anatomy, from representatives of the main groups of Australian native rodents.

Materials and Methods

Animals: Rodents used in the present study were obtained from the following sources:

The hopping mice (*Notomys alexis*) and plains mice (*Pseudomys australis*) were derived from a laboratory stock maintained at the Medical School, University of Adelaide (see Breed 1975).

The water rat (*Hydromys chrysogaster*) was obtained from the River Torrens near Adelaide; *Rattus fuscipes greyi* was collected near Stirling, S.A., *Pseudomys hermannsburgensis* was laboratory bred from parents collected near Curtin Springs, N.T., and *Zyzomys argurus* and *Zyzomys woodwardi* were collected near Darwin by Dr R. Begg.

Material from the following species was obtained from animals held at the Institute of Medical & Veterinary Science field station in Adelaide: *Pseudomys higginsi, P. apodemoides* (see Baverstock *et al.* 1977a for specific terminology), *P. nanus, Conilurus penicillatus, Leporillus conditor, Melomys littoralis, M. cervinipes, Uromys caudimaculatus, Rattus leucopus leucopus, R. sordidus, R. colletti, R. lutreolus* and *Notomys mitchellii* (for details of sites of capture see Baverstock *et al.* 1977b, 1977c, Robinson *et al.* 1978). Nomenclature *of Rattus* spp. used is that of Robinson *et al.* 1978.

Preparation of spermatozoa: Spermatozoa from 1 *Hydromys chrysogaster*, 4 *M. littoralis*, 6 *N. alexis*, 1 *P. apodemoides*, 4 *P. australis*, 1 *P. hermannsburgensis*, 3 *R. fuscipes greyi* and 4 *Z. argurus* were obtained immediately after killing the animals with chloroform. The tail of one epididymis and adjacent vas deferens was dissected out and sperm droplets squeezed onto several slides. Thin smears were made by using the edge of another slide. Spermatozoa from the other species were obtained after anaesthetising the animals with urethane. A small incision was then made in one scrotal sac and part of the tail of one epididymis was removed from which sperm smears were obtained as described above. After allowing the smears to dry, they were flooded with 2.5% glutaraldehyde in 0.01M sodium cacodylate fixative and a coverslip placed on top which was fixed in position with De Pe X to give a semi-permanent mount. Latterly wet smears were fixed with either glutaraldehyde or picric acid/glutaraldehyde/formaldehyde mixture (see Ito & Karnovsky 1968).

Methods of assessment of spermatozoa: Smears were inspected by phase contrast and spermatozoa that appeared intact, straight, and reasonably well isolated, were selected for measuring. Using an eyepiece micrometer the following measurements were made: (1) head length from the most caudal part of head to top of the curve (see Braden 1959), (2) length of midpiece, and (3) length of remainder of tail (usually the principal and end pieces were not well differentiated, so they were included together as one measurement). Several spermatozoa from each individual were observed and usually the measurements were similar or identical. When some variation occurred the range has been included (Table 1).

Smears were also observed by Nomarski differential interference microscopy, and selected spermatozoa photographed. Measurements obtained by phase contrast were compared with those made from photographs obtained by Nomarski.

Attempts were made to determine the presence of the acrosome and the distribution of DNA in the sperm head from *P. australis, M. littoralis, N. alexis, Z. argurus* and *H. chrysogaster*. The DNA was determined according to the Feulgen method (Pearse 1968) and by the use of DAPI (Russell *et al.* 1975). After staining by the Feulgen method smears were observed by epifluorescence using green excitation (Ploem 1967) (Olympus excitation filter IF 545, with a G dichroic mirror and barrier filter Y595) and by normal bright field microscopy. When DAPI in distilled water (about 0.001%) was used the filter system included ultraviolet excitation (UG 1), U dichroic mirror, and Y455 barrier filter. Acridine orange was used in an attempt to visualise the acrosome by fluorescence microscopy (see

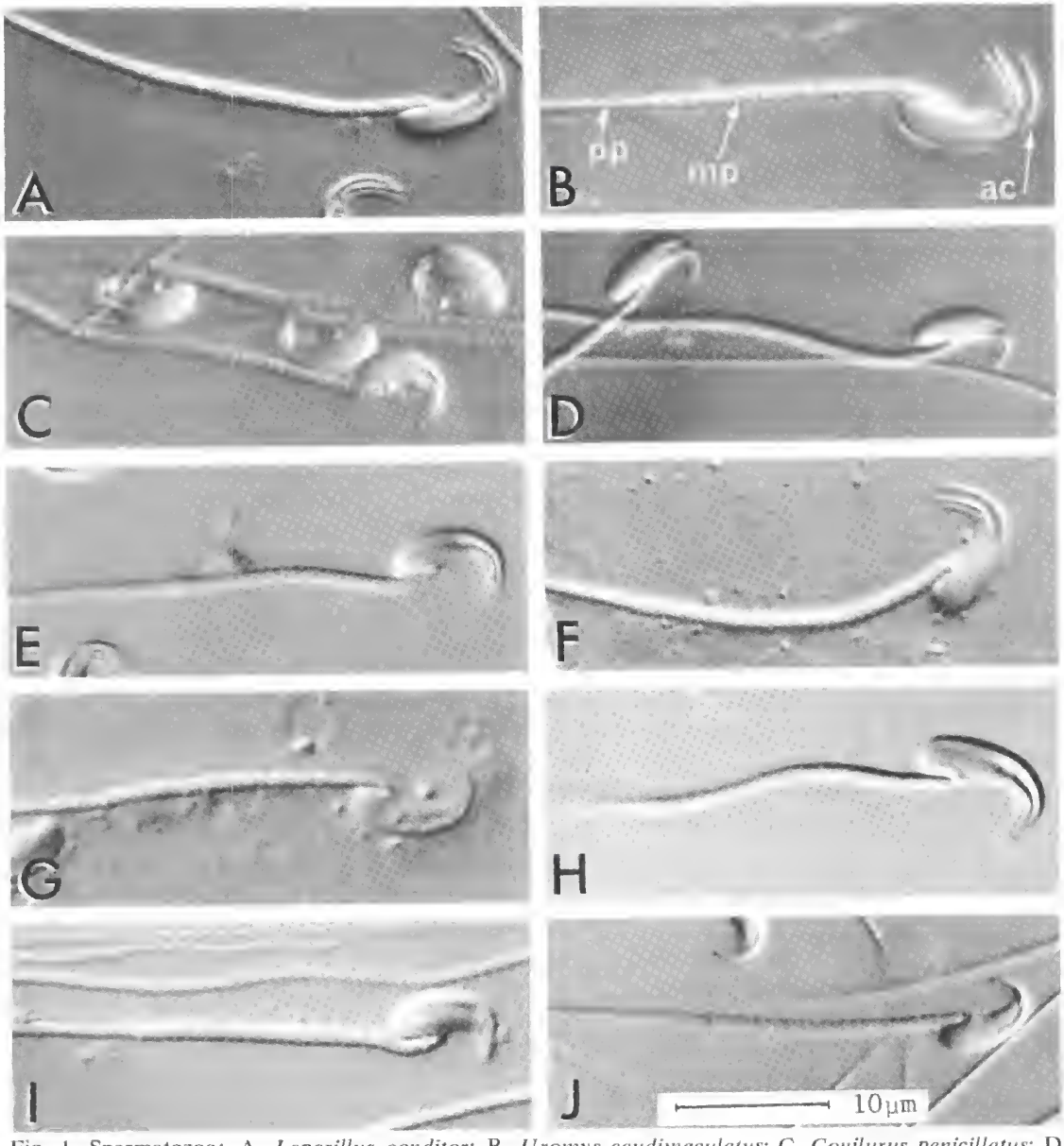

Fig. 1. Spermatozoa: A. *Leporillus conditor*; B. *Uromys caudimaculatus*; C. *Conilurus penicillatus*; D. *Zyzomys argurus*; E. *Pseudomys higginsi*; F. *P. hermannsburgensis*; G. *P. nanus*; H. *P. australis*; I. *P. apodomoides*; J. *Hydromys chrysogaster*. mp = midpiece pp = principal piece ac = acrosome

Bishop & Walton 1960). For this, blue exeitation (BG 12), B diehroic mirror, and Y455 barrier filter were used.

Body, testis and accessory organ weights: Some of the animals from which spermatozoa were obtained were weighed and one testis, seminal vesieles together with coagulating glands if present, and ventral prostates were also removed and weighed after removing adherent fat from the organs. The weight of a single testis was doubled to give an approximate weight of the paired testes.

Results

Spermatozoal morphology

Figs 1 & 2 and Table 1 show the morphology of the head and mid-piece of spermatozoa from the various speeies. Intra-individual

TABLE 1

Comparative head and tail lengths of spermatozoa from various Australian native rodents.

Species	Size of spermatozoa (μm) Length of head*	Mid-piece	Principal and end piece	Total length
Conilurus penicillatus	7	20–22	96	123–125
Hydromys chrysogaster	7	20	88	115
Leporillus conditor	9	23	78	110
Melomys littoralis	8	22	80	110
Notomys alexis	5–8	25–27	70	100–105
N. mitchellii	9	24	65	98
Pseudomys apodemoides	8	22	90	120
P. australis	9	23	88–93	120–125
P. hermannsburgensis	8–10	23	85	116–118
P. higginsi	8	20–22	70–85	98–115
P. nanus	9	22	96	127
Rattus colletti	12	51	95	158
R. fuscipes greyi	12	48	102	162
R. l. leucopus	12	135		147
R. lutreolus	13–15	54	95	162–164
R. sordidus	12	45–50	95	152–157
Uromys caudimaculatus	8–10	20	72–82	100–112
Zyzomys argurus	7	22	108	137

* From base to top of curvature of hook.

variation was small except for sperm from *Notomys alexis* and *N. mitchellii*.

Sperm from all species, apart from *Rattus* spp. and *Notomys* spp. conformed to the same general pattern. The sperm head had a fairly broad base which tapered to two or three prongs or hooks. The top hook was usually larger and invariably single, whereas the lower one was often bifid. There was inter-specific variation in head length (Table 1). Staining with Feulgen and DAPI demonstrated that the top prong consisted of DNA, and Acridine orange indicated the presence of an acrosome covering the nuclear material on top of the hook and extending beyond its tip. The lower hook(s) appeared to have DNA only at the base and no orange or red colour was obtained with Acridine orange.

About one-third the way up the ventral side of the sperm head a small spike occurred to which is attached the connecting piece. On the dorsal side, a ridge could sometimes be seen which stained orange with Acridine orange, and presumably represent the continuation of the acrosome down the dorsal side of the head. The midpiece had very prominent gyres of mitochondria.

Species that conform to the above general pattern and had three hooks included *Leporillus conditor, Pseudomys hermannsburgensis, P. australis, P. higginsi, P. nanus, P. apodemoides, Conilurus penicillatus, Melomys littoralis, M. cervinipes* and *Uromys caudimaculatus*. These spermatozoa were similar except that the length of the head of *C. penicillatus* was shorter. *Melomys littoralis* and *M. cervinipes* had smaller hooks, and only on close examination were three discernible. *Zyzomys argurus, Z. woodwardi* and *Hydromys chrysogaster* had spermatozoa of the same basic structure but the hooks were not so long and only two were visible. No bifid lower prong could be seen. The sperm heads tended to be shorter than most of those with the three hooked sperm, and the breadth of the sperm head was also less. *H. chrysogaster* also had a relatively short midpieee.

The spermatozoa from *Notomys alexis* were variable but consistently different. Fig. 2 shows three different morphological types. The head length was generally short and there was usually a short top hook and a very truncated lower hook. Only the top hook appeared to be surrounded by an acrosome. The midpiece of *N. alexis* was generally longer than that for other species described above (see Table 1), but the principal/end piece appeared shorter. *N. mitchellii* also had intra-individual variable spermatozoal morphology. Sometimes there was a single top hook which was longer than in *N. alexis* and a short truncated lower hook, whereas on other occasions, two or three straight short hooks occurred.

The spermatozoa of all *Rattus* species were markedly different from those described and generally appeared similar to each other and to *R. norvegicus* and *R. rattus* (Friend 1936). The heads were long and attenuated with a long sharp hook. Acridine orange demonstrated the acrosome primarily on the top surface of the sperm head and extending beyond the DNA to the tip of the hook. The junction between the mid and principal piece was not easily visible, in contrast to the situation in the previous species described, but when visible it appeared that the midpiece was at least twice as long as that for sperm from the other groups of Australian rodents. Since the principal and end pieces were generally similar

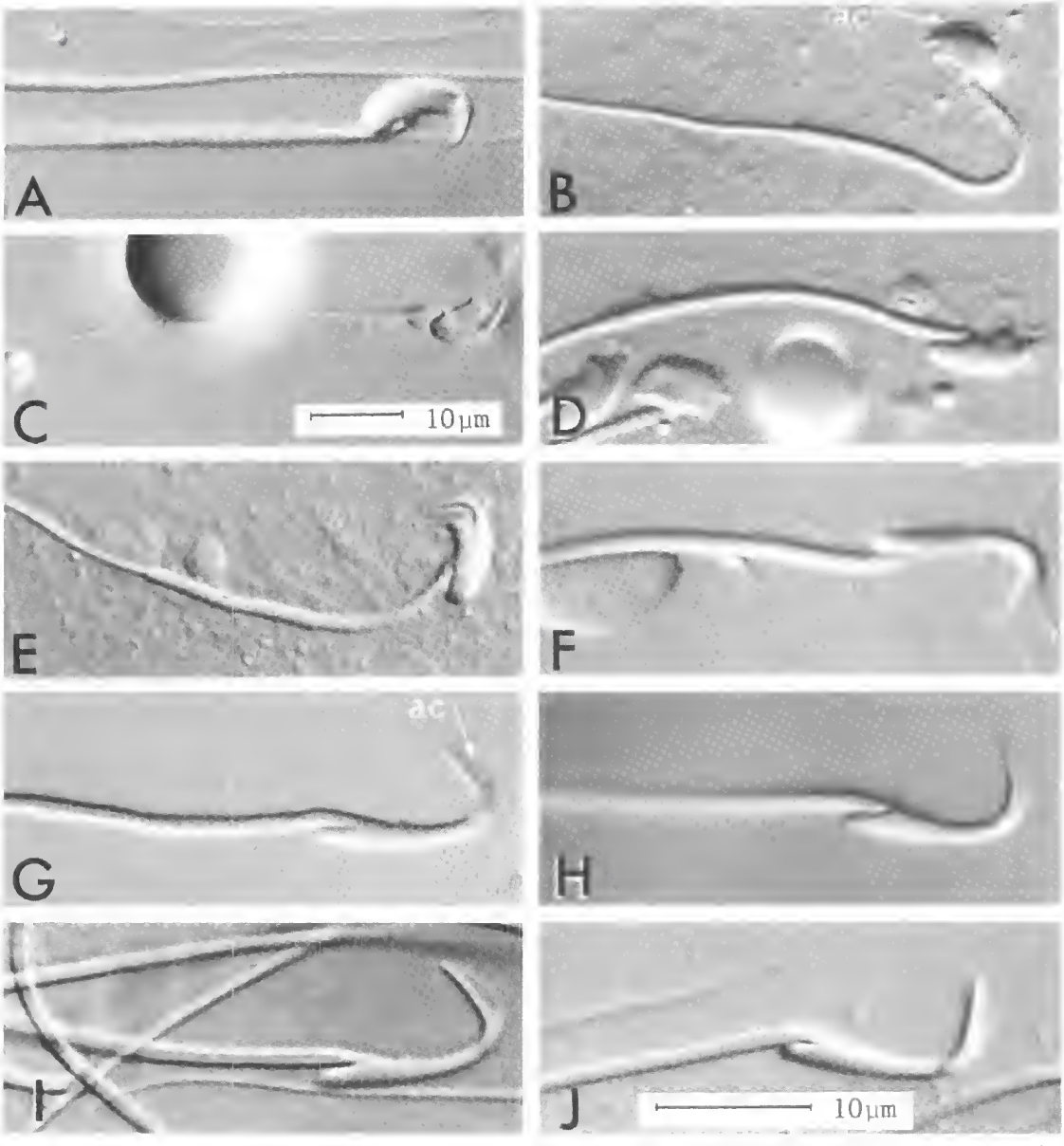

Fig. 2. Spermatozoa: A. *Melomys littoralis;* B. *Notomys alexis*; C. *N. alexis*; D. *N. alexis*; E. *N. mitchellii*; F. *Rattus sordidus*; G. *R. colletti*; H. *R. l. leucopus*; I. *R. fuscipes greyi*; J. *R. lutreolus.*

in lengths to those of the other groups, except for *N. alexis,* the resultant total length of the sperm was considerably greater.

Testis and male accessory organs

Analysis of gonadal weights has been performed on some of the species of animals that yielded motile sperm. Table 2 demonstrates that testis weight/g body weight was similar in *P. australis, R. fuscipes greyi, M. littoralis* and *H. chrysogaster* in spite of the considerable range of absolute body weights (60–540 g). The relative testis weight of *Z. argurus* was somewhat less and those of *N. alexis* and *N. mitchellii* were markedly lower than in the other species examined (Table 2).

The testes of *P. australis, R. fuscipes, M. littoralis, H. chrysogaster* and *Z. argurus* invariably occurred in a scrotal sac with the tail of the epididymis protruding into an extension of this towards the body wall of the scrotum.

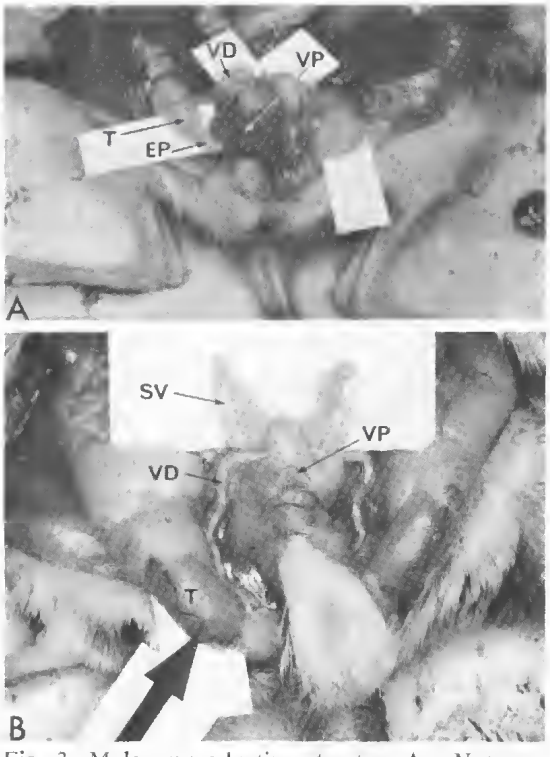

Fig. 3. Male reproductive tracts: A. *Notomys alexis;* B. *Zyzomys argurus.* T = testis, VD = vas deferens, SV = seminal vesicle, EP = tail of epididymis, VP = ventral prostate.

By contrast, the testes of *N. alexis* and *N. mitchellii* appeared to usually be naturally cryptorchid and lay in the abdomen close to the body wall ventral to the tail. Externally the skin of *Notomys* species (and other species) was usually pigmented and only sometimes a slight swelling occurred. The tail of the epididymis lay in a small cremastic sac.

The relative weights of the male accessory organs of *R. fuscipes, M. littoralis, H. chrysogaster,* and *P. australis* were similar. Seminal vesicles, together with coagulating glands when present, ranged from 0.4 to 1.8% of total body weight, and those for ventral prostates 0.06–0.31%. The relative weights of seminal vesicles and coagulating glands for *Z. argurus* were somewhat less (0.3%–0.5%), although that of the ventral prostate was similar. The morphology of the seminal vesicles of *Z. argurus* (Fig. 3) differed somewhat from that of the other species. *N. alexis* and *N. mitchellii* had seminal vesicles that were only just visible to the naked eye. They measured about 3 mm in maximum diameter. Coagulating glands could not be found on macroscopic dissection, but the relative weights of the ventral prostates were considerably greater than those for the other species examined except for *R. fuscipes.* Development of the ventral prostate occurs rapidly at around the time of puberty

TABLE 2

Body and male reproductive organ weights of some Australian native rodents.

Species	No. of animals	Body wt (g)	Testis wt (mg)	Seminal vesicle and coagulating gland wt (mg)	Ventral prostate wt (mg)
Hydromys chrysogaster	1	536	12460*** (2.3%)	2378 (0.4%)	**
Melomys littoralis	3	61 ± 3	1684 ± 62 (2.6–3.0%)	700 ± 14 (1.0–1.3%)	63 ± 9 (0.08–0.1%)
Notomys alexis	4	29 ± 2	33 ± 9 (0.17–0.07%)	—*	97 ± 20 (0.20–0.46%)
N. mitchellii	2	33 ± 2	61 ± 5 (circa 0.2%)	—*	79 ± 3 (c. 0.2%)
Pseudomys australis	3	59 ± 6	1739 ± 362 (3.0–4.5%)	1000 ± 94 (1.4–1.8%)	60 ± 9 (0.06–0.16%)
Rattus fuscipes	3	100 ± 21	4410 ± 231 (3.4–6.1%)	1270 ± 186 (1.0–1.6%)	247 ± 38 (0.29–0.31%)
Zyzomys argurus	3	53 ± 11	400 ± 49 (0.4–1.1%)	185 ± 27 (0.3–0.5%)	43 ± 3 (0.07–0.13%)

* Seminal vesicles and coagulating glands are vestigeal in *Notomys* species. Maximum diameter of about 3 mm.
** Not weighed.
*** Range of ratios of organ weights to total body weights expressed as percentage.

(Breed 1979) and is therefore likely to be androgen dependant.

Discussion

Spermatozoa from the Australian rodents investigated fell into three groups. Those from single species of *Conilurus*, *Leporillus*, *Uromys*, *Hydromys*, the two species of *Zyzomys* and *Melomys* and the five species of *Pseudomys* were all similar to each other. Most had a sperm head with three hooks, and observations of only two may reflect problems of technique. The top hook was invariably made up of DNA over which an acrosome occurred, whose material stained orange with Acridine orange. This appears to coincide with the hook observed in many other murid rodents (e.g. Friend 1936; Bishop & Walton 1960). However, the lower, usually bifid, hook appears to be a unique character occurring in Australian rodents and is presumably derived. This hook appears to only have DNA at its base, and is likely to be made up mainly of acrosomal material, even though it does not stain orange or red with Acridine orange. Variability of staining of acrosomal material with Aridine orange has previously been documented (Allison & Hartree 1970). It seems likely, therefore, that all the above genera have been derived from the same ancestral form in which a two or three-hooked sperm evolved.

The sperm morphology of *Notomys* species is, however, markedly different. In *N. alexis* and *N. mitchellii* there was much individual variability in sperm morphology. Generally, however, the sperm of *N. alexis* was characterised by a single small truncated top hook. Using Nomarski optics, it appeared that this was covered by an acrosome which did not, however, appear to fluoresce orange or red with Acridine orange. The lower hook in this species was short, truncated, and at times barely recognisable, and thus may represent the DNA staining area of the lower hooks occurring in the other genera. In *N. mitchellii* a longer top hook was visible and this sometimes appeared to be the only well-defined hook. Most sperm had three hooks (as in the Pseudomyinae and other groups) but they appeared shorter with a sharper angle of curvature. It is likely, therefore, that the lack of well defined hooks in *N. alexis* is a secondarily derived form from an ancestral two or three pronged Pseudomyid-like sperm. In *N. alexis* the total length of the head and the relative proportions of the midpiece to the rest of the sperm tail was also considerably different from all the other genera. In *Notomys*, in contrast to the other species studied, there appeared to be relatively few spermatozoa in the epididymis and vas deferens, the ratio of testis weight/total body weight was markedly less, the testes usually appeared naturally cryptorchid, the seminal vesicles and coagulating glands markedly smaller and the ventral prostate relatively larger. Vaginal plugs, after recent matings, have not yet been observed in *N. alexis*. This may be due to lack of development and secretion of the seminal vesicles and coagulating glands. Other physiological and behavioural significances of these differences have yet to be elucidated, but the smaller testes and few stored sperm suggest only infrequent matings would result in successful fertilizations. The social-sexual behaviour of these species is not known in detail but it appears that *Notomys alexis* is a highly social animal (Stanley 1970). An anatomical feature that may be related to this is the occurrence of prominent chest glands (Stanley 1970, Watts 1975). We therefore suggest that *Notomys* has diverged further from the basic pseudomyid stock than suggested on morphological characters by Tate (1951). Further studies on spermatozoal and male reproductive tract morphology of the other *Pseudomys* and *Notomys* species should be carried out to determine if our findings are characteristic of the genera. This may not be the case as Illison[1] (1971) states that not all *Pseudomys* species have spermatozoa of similar morphology, although she considers that only two hooks are normally present. She claimed that spermatozoa of *P. shortridgei* represented the primitive sperm type and *P. delicatula* (as *Leggadina delicatula*) sperm had no hooks. Unfortunately we have not been able to reinvestigate these findings.

The spermatozoa of *Rattus* spp. were very different from those of all other Australian rodents and similar to congeners occurring on other continents. Illison[1] came to a similar conclusion. This suggests an independent line of evolution and invasion into Australia of *Rattus*. Lidicker (1968) suggested, from comparative morphological studies of the penis, that there were two rodent invasions into New Guinea—one that gave rise to all rodents

[1] Illison, L., Abstract presented at Aust. Mammal Society Meeting, Vol. 2, No. 8, December, 1971.

except *Rattus* and the other that gave rise to the "native" species of *Rattus*. More recently Baverstock *et al.* (1977b, 1977c), from chromosomal data, concluded similarly for the Australian rodents. Our data on sperm morphology therefore supports the phylogenetic conclusions of these authors, but conflicts with those of Tate (1951) who regarded the Hydromyinae as a separate subfamily and Simpson (1961) who regarded the Hydromyinae and Pseudomyinae (excluding *Rattus*) as separate subfamilies.

The significance of interspecific differences in sperm morphology has been discussed by Friend (1936), Fawcett (1970, 1971, 1975, 1977) and others. Some mammal spermatozoa have large acrosomes, e.g. guinea pigs (Fawcett 1970) and musk shrews (Green & Dryden 1976). The latter relate this to the thick corona radiata around the eggs. Acrosomes of spermatozoa of the Pseudomyinae/Hydromyinae/*Uromys*/*Melomys* stock were not very well developed, whereas those of the *Rattus* spp. were similar to that of the laboratory rat.

The sperm head is very rigid, which may be necessary for penetration of the thick zona around the egg (Bedford & Calvin 1974), whereas the hook of murid sperm may be involved in motility (Cohen 1977). However, head shape does not appear to be closely related to species specificity for penetration of the oocytes, as human sperm can penetrate hamster eggs (Rudak *et al.* 1978). Fawcett (1977) has suggested that the hook may deflect the sperm from the surface of the mucosal lining in the oviduct but as yet there appears to be no evidence for this.

The midpiece also differs greatly between species. Occurrence of increased mitochondrial development of the midpiece correlates with the evolution of internal fertilization (Afzelius 1971; Fawcett 1978), but variation in number and shape of mitochondria between species of mammals has not yet been given any satisfactory explanation. There is no obvious correlation between number of mitochondria and the distance sperm have to swim to bring about fertilization. Thus although there is as yet no agreed explanation for either sperm head shape or midpiece length in mammalian spermatozoa, these characters may be useful in determining phylogenetic similarities and differences when taken into consideration with other morphological, biochemical, cytological and behavioural characteristics.

Acknowledgments

We should specially like to thank Drs Chris Watts and Peter Baverstock for making available to us many of their valuable rodents and for criticising the manuscript, Dr Begg for supplying us with *Zyzomys* spp., Dr Possingham of CSIRO for permitting us the use of his Nomarski microscope, Mr R. Murphy, Mrs J. Brazier and Mrs B. Sheldon for assistance in preparing the manuscript. We should also like to thank the National Parks & Wildlife Services for permits to catch *Hydromys* and *Rattus fuscipes* in South Australia and *Notomys alexis* and *Pseudomys hermannsburgensis* in the Northern Territory.

References

Afzelius, B. A. (1971) Sperm morphology and fertilization biology. *In* R. A. Beatty and S. Gluecksohn-Waelsch (Eds) "Edinburgh symposium on the genetics of the spermatozoon."

Allison, A. C. & Hartree, E. F. (1970) Lysosomal enzymes in the acrosome and their possible role in fertilization. *J. Reprod. Fert.* **21**, 501-515.

Baverstock, P. R., Watts, C. H. S. & Cole, S. R. (1977a) Electrophoretic comparisons between allopatric populations of five Australian Pseudomyinae rodents (*Muridae*). *Aust. J. Biol. Sci.* **30**, 471-485.

Baverstock, P. R., Watts, C. H. S. & Hogarth, J. T. (1977b) Chromosome evolution in Australian rodents I. The Pseudomyinae, the Hydromyinae and the *Uromys*/*Melomys* group. *Chromosoma* **61**, 95-125.

Baverstock, P. R., Watts, C. H. S. & Hogarth, J. T., Robinson, A. C. & Robinson, J. F. (1977c) Chromosome evolution in Australian rodents II. The *Rattus* group. *Ibid.* **61**, 227-241.

Bedford, J. M. & Calvin, H. I. (1974) The occurrence and possible functional significance of — S — S — cross links in sperm heads, with particular reference to eutherian mammals. *J. exp. Zool.* **188**, 137-156.

Bishop, M. W. H. & Walton, A. (1960) Spermatogenesis and the structure of mammalian spermatozoa. Chapter 7. *In* A. S. Parkes (Ed.) "Marshall's Physiology of Reproduction", Vol. 1 (2). (Longman, London.)

Braden, A. W. H. (1959) Strain differences in the morphology of the spermatozoa of the mouse. *Aust. J. Biol. Sci.* **12**, 65-71.

Breed, W. G. (1975) Environmental factors and reproduction in the female hopping mouse (*Notomys alexis*). *J. Reprod. Fert.* **45**, 273-281.

Breed, W. G. (1979) The reproductive rate of the hopping mouse (*Notomys alexis*) and its ecological significance. *Aust. J. Zool.* (in press).

Cohen, J. (1977) "Reproduction." (Butterworths, London.)

FAWCETT, D. W. (1970) A comparative view of sperm ultrastructure. *Biol. Reprod. Suppl.* **2**, 90-127.

FAWCETT, D. W. (1975) The mammalian spermatozoon. *Dev. Biol.* **44**, 394-436.

FAWCETT, D. W. (1977) Unsolved problems in morphogenesis of the mammalian spermatozoon. *In* R. P. Brinkley & K. R. Porter (Eds) "International Cell Biology," pp. 588-601. (Rockefeller Univ. Press, New York.)

FAWCETT, D. W., ANDERSON, W. A. & PHILIPS, D. M. (1971) Morphogenetic factors influencing the shape of the sperm head. *Dev. Biol.* **26**, 220-251.

FRIEND, G. F. (1936) The sperms of British muridae. *Q. J. Micros. Sci.* **78**, 419-443.

GREEN, J. A. & DRYDEN, G. L. (1976) Ultrastructure of the epididymal spermatozoa of the Asiatic musk shrew *Suncus murinus*. *Biol. Reprod.* **14**, 327-331.

ITO, S. & KARNOVSKY, M. J. (1968) Formaldehyde-glutaraldehyde fixatives containing trinitro compounds. *J. Cell Biol.* **39**, 168A-169A.

LIDICKER, W. Z. (1968) A phylogeny of New Guinea rodent genera based on phallic morphology. *J. Mammal* **49**, 609-643.

PEARCE, A. G. E. (1968) "Histochemistry: theoretical and applied" (3rd Edit.) Vol. I. (Churchill, London.)

PLOEM, J. S. (1967) The use of a vertical illuminator with interchangeable dichroic mirrors for fluorescent microscopy with incident light. *Z. Wiss. Mikros* **68**, 129-140.

ROBINSON, J. F., ROBINSON, A. C., WATTS, C. H. S. & BAVERSTOCK, P. R. (1978) Notes on rodents and marsupials and their ectoparasites collected in Australia in 1974/75. *Trans. R. Soc. S. Aust.* **102**, 59-70.

RUDAK, E., JACOBS, P. A. & YANAGIMACHI, R. (1978) Direct analysis of the chromosome constitution of human spermatozoa. *Nature* **274**, 911-913.

RUSSELL, W. C., NEWAN, C., WILLIAMSON, D. H. (1975) A simple cytochemical technique for demonstration of DNA in cells infected with mycoplasmas and viruses. *Ibid.* **253**, 461-462.

SIMPSON, G. G. (1961) Zoogeography of Australian Mammals. *Evolution* **15**, 431-446.

STANLEY, M. (1970) An ethogram of the hopping mouse, *Notomys alexis*. *Zeit. für tierpsychiol.* **29**, 225-258.

TATE, G. H. H. (1951) Rodents of Australia and New Guinea. Results of Archbold Expedition, 65. *Bull. Amer. Mus. Nat. Hist.* **97**, 183-430.

WATTS, C. H. S. (1974) The native rodents of Australia: a personal view. *Aust. Mam.* **1**, 109-116.

WATTS, C. H. S. (1975) The neck and chest glands of the Australian hopping mice. *Aust. J. Zool.* **23**, 151-157.

ASPECTS OF GROWTH AND FEEDING IN GOLDEN CARP, CARASSIUS AURATUS, FROM SOUTH AUSTRALIA

BY B. D. MITCHELL

Summary

Age and growth were determined in populations of Carassius auratus from the River Murray, Millbrook Reservoir, and a farm dam. Fish from Millbrook grew most rapidly, reaching 13.1 cm at the end of the first year's growth. The Uraidla population exhibited the lowest growth rate, reaching 4.7 cm at the end of the first year. Significant differences in length-weight relationships occurred between all populations. The length (l) –weight (w) equations were: Millbrook, $w = 0.029l^{3.141}$ ($r^2 = 0.989$); Cobdogla, $w = 0.014l^{3.265}$ ($r^2 = 0.923$); Uraidla fish (< 6 cm), $w = 0.024l^{3.302}$ ($r^2 = 0.950$), Uraidla fish (> 6 cm), $w = 0.054l^{2.759}$ ($r^2 = 0.908$).